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ATTENTION: Vittorio Sgaramella 2/5/93
Dept. of Genetics and Microbiology
University of Pavia

From: Greg Tombline
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New York, USA

Dear Vittorio,

Thanks for your encouragement in your last letter - things have been somewhat of an uphill climb lately. Our experiments are moving slowly but surely.... I obtained some more 32P dATP to do the fingerprinting this week, did the reactions and loaded them on the old type of gel (6% acrylamide/bis as the cross linker) and have given up using the Hydrolink. This time the gel was much easier to handle, and dried without a wrinkle or rip. The fingerprinting looked OK after 7 hours exposure without an intensifying screen at room temp, but since the sequencing was done with 35SdATP, it had not shown up yet. The gel is currently being exposed for longer. I ran it about twice as long in order to resolve the area of the mutants well, but it was slightly blurry. We have been forced to carry on with another power supply which Ken borrowed from the fifth floor which sometimes has difficulty running at constant current. Anyway, the worst scenario would consist of running another gel, which I will do this weekend.... and borrow Miklos Muller's power supply in the process.....

I have not been able to delve into the endonuclease problem... I really wanted to knock of the fingerprinting and have been busy in David's arena as well. I did try to locate the gel which you requested a longer exposure, and thought that I had found it- but with no avail. I will try a couple other gels.... exposing them over the weekend at -70 with an intensifying screen but I can't promise. I might have discarded it since I thought that the exposure we had would suffice.... ie... the one that I took to the media services (I'll photocopy and fax along with this letter the print of the gel which I think you mean). Also - as soon as the fingerprinting is clear, I'll look back at locating the specifics/ conditions of the endonuclease...

I also wanted to mention a couple more things in regard to the fingerprinting- I exposed and scanned a recent gel (which had been wrinkled at the ponits of mutaion) on the phosphorimager and analyzed individual lanes by integrating the counts over the whole lane and printing out the peaks/etc..... in order to determine the hot spots. The results are curious- the hot spots for some seem strikingly periodic- I'll enclose one so you can see what I mean. I am hoping to use the method to compare the counts distributed across each lane and compare the lanes. In relation to this, I sat down and diagrammed out the sequece of the respective regions for PIII and P4 and highlighted possible stops (I'll enclose this also).... when we get the fingerprint which should be within a few days with some luck, I'll mark the experimentally seen stops so we can have a real picture. Hang in there...

Mike told me that you have been sending me some Email, but as of now I don't have an account- I will get one on Monday.... also - Mike is on jury duty this week so communication has not been the best. I'll give it a shot ASAP.

I'll be in touch very soon. Hope that all is well..... Ciao,

GREG.

PS: THERE ARE 8 PAGES TO THIS FAX...